Behavioral video coding analysis of chronic morphine administration in rats

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Received April 5, 2024; Accepted August 20, 2024

DOI: 10.3892/br.2024.1856

Abstract. The present study assessed the behavior of morphine-addicted rats using behavioral video coding technology, to evaluate effective methods for identifying morphine addiction. Rats were divided into a control group (n=15) and a morphine addiction group (n=15). The morphine addiction model was established with a 14-day increasing dose scheme, confirmed using a conditional place preference (CPP) experiment. After successful modeling, the rats' behavior was recorded for 12 h, then coded and analyzed using Observer XT behavior analysis software. Compared with the control group, morphine-addicted rats showed increased heat pain tolerance time (P=0.039) and spent more time in the white box during the CPP experiment (P<0.001). Video coding analysis revealed significant behavioral changes in morphine-addicted rats compared to controls. In addition to being lighter, morphine-addicted rats showed decreased water intake, reduced licking of forelimbs and hind limbs, and altered sleeping posture (sleeping curled up) during the day (all P<0.05). In conclusion, chronic morphine administration in rats leads to distinctive behavioral changes, including decreased licking frequency, reduced water intake and altered sleep posture. Video coding analysis, as a safe and non-invasive method, may provide a convenient and efficient approach for studying morphine addiction in rats.

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Key words: morphine, addiction, rat, conditional place preference experiment, video coding

Introduction

Morphine, a typical opioid, is used in clinical settings to relieve cancer-related pain, especially in patients with bone cancer (1-4). However, addiction and dependence remain significant issues that affect its clinical use (5-8). In the context of social and public health, drug abuse and addiction are major public health concerns (9-12). Addiction is currently viewed as a chronic and relapsing disorder (13-15). The process of drug addiction engages reward-related learning and memory systems, showing synaptic plasticity within these systems (16-28). The mechanisms behind opioid addiction are closely related to the central dopamine reward neural pathway, mainly involving the prefrontal cortex (29), the nucleus accumbens (30-32), and the ventral tegmental area (33-38). Exploring the neurobiological mechanisms of drug addiction is crucial for clinical treatment.

Research on opioid addiction mechanisms often focuses on the morphine dependence model in rodents, usually rats or mice. The success of this model is assessed through conditioned place preference (CPP) or self-administration (SA) experiments (39-43). However, these methods are time-consuming, labor-intensive, require long-term behavioral training of animals and are subject to experimenter bias. CPP is an experimental tool for evaluating drug-seeking behavior or psychological craving in animals, requiring the association of a rewarding stimulus with a non-rewarding conditioned stimulus to confirm the presence of a rewarding stimulus. This process is tedious, time-consuming and requires a large sample size to avoid errors. SA experiments are the standard used for verifying drug addiction but need specific environments and equipment, and trained personnel. Additionally, issues such as hemorrhage, trauma, infection and death due to intravenous intubation in animals can affect the experimental results and process (44).

To the best of our knowledge, there have been no previous reports on the use of behavioral video coding analysis for morphine addiction. Behavioral video analysis technology may provide a convenient and efficient method for studying addictive behaviors. Recording videos of animals during the addiction modeling process and then coding these behaviors (45) allows for the identification of characteristic

behavioral changes following morphine addiction in rats. Therefore, the present study assessed the behavior of morphine-addicted rats through behavioral video analysis and to evaluate this potentially efficient method to verify morphine addiction model in rats, offering a new approach for future studies on drug addiction behavior.

Materials and methods

Animals. Sprague-Dawley (SD) rats were purchased from SPF Biotechnology Co., Ltd. Rats were housed in a temperature-controlled environment (21-22˚C) within an animal holding room, following a 12 h light/dark cycle. Food and tap water were available *ad libitum*. The morphology, behavior, diet and water intake of the rats were observed at 8:00 a.m. and 8:00 p.m. each day. The body weight of the rats was measured every day before morphine injection. The experiment lasted a total of 22 days. Rats were acclimated to the laboratory for 1 week (day -6 to day 0) before starting administration. Chronic morphine administration and CPP assessment were performed in rats from day 1 to day 14, with video recording of rat behavior was carried out on day 15. The ethics committee of Beijing Institute of Basic Medical Sciences (Beijing, China; approval no. IACUC-DWZX-2022-715) approved the experimental protocol.

The experiment would be terminated in the event of any serious harm to animal welfare (including but not limited to): i) Loss of 15-20% of body weight; ii) infection in brain area; iii) other indicators such as pain, respiration (severe respiratory tract infection, dyspnea, cyanosis and other phenomena) and appearance (severe muscle atrophy and non-healing wounds). No rats met the criteria for early euthanasia during the course of the present study. After completing the experiment, the rats were placed into a non-precharged chamber and euthanized by CO_2 inhalation (30% vol/min). Death was confirmed by cardiac and respiratory arrest and dilated pupils.

Drugs: Morphine hydrochloride injection (10 mg/ml, Shenyang First Pharmaceutical Factory, Shenyang) was diluted with sterile saline to achieve a 2.5 mg/ml working solution.

Apparatus. A Hot and Cold Plate Plantar Analgesia Instrument (cat. no. 28-0010; Shenzhen Huayang Biotech Co., Ltd.) was used to assess the thermal pain response of rats. The device included a transparent plastic chamber (25 cm diameter, 60 cm height) and a heating plate. Rats were placed in the plastic chamber, and the heating plate temperature was kept at 40±1˚C using a thermal probe and electronic feedback circuit. The timer started when the rats were placed in the chamber and was stopped manually when the rats showed behaviors such as lifting their hind limbs or licking their forelimbs. If no thermal response was observed within 3 min, the timer was stopped and the rats were promptly removed.

A Xiaomi Smart Video Camera PTZ Version 2K (cat. no. MJSXJ09CM; Xiaomi Inc.) was placed in the rat cage for video recording. A wooden board with a 5 cm diameter circular hole was positioned between the camera and the rat.

For the CPP experiment, a CPP apparatus was used, consisting of two compartments (30x60x30 cm; Zhongshi Technology). The different compartments had distinct floors and walls, separated by a removable plastic board. One compartment had black and white striped walls and a white floor, while the other had black and white checkered walls and a black floor. The test chamber was set up under 40 lux dim lighting and shielded from white noise (46).

The Observer XT (Noldus Information Technology BV) software was used for coding and analyzing recorded rat videos, focusing on typical rat behaviors (45).

Morphine administration. The morphine dependence model was established in 6-week-old (weight, 180-200 g) SD rats through a 14-day continuous dose escalation protocol. Male (n=15) and female (n=15) rats were randomly divided into two groups (47-52). The morphine group received intraperitoneal morphine at a dose of 5 mg/kg on day 1, while the control group received an equivalent volume of normal saline. The morphine dose increased gradually, reaching 100 mg/kg on day 14 for the morphine-addicted rats, while the control rats continued to receive an equivalent volume of normal saline (53-57). The initial dose of morphine group was 5 mg/kg on the first day, and the dose gradient was 5 mg/kg per day from day 2 to day 7, 10 mg/kg per day from day 8 to day 14, and reached 100 mg/kg on day 14 (53-65). The half-life of morphine in rats is generally 3-4 h, the drug effect of morphine lasts for 4-6 h and morphine is largely metabolized in 8-10 h (66-69). Morphine was administered daily at 8 am and 8 pm, ensuring an interval of about 12 h between doses (70).

Plantar heat tolerance test. Prior to each intraperitoneal injection of morphine, the time from the beginning of exposure to the hot plate at 40˚C to the lifting or licking of the hind foot was measured. The maximum measurement time was limited to 3 min. The same measurement was taken 1.5 h after the intraperitoneal injection of morphine under the same conditions, recording the time to foot lifting or licking.

CPP Test. The CPP experiment was divided into two phases, the pre-experimental phase and the test phase (46). The pre-experimental phase lasted four consecutive days, with two sessions conducted daily. Rats (n=10) were randomly selected from each of the morphine and control groups, resulting in a total of 20 rats. Each session lasted 15 min. Conditioned reflexes were established by confining the rats to a white drug delivery box (referred to as the white box) for 15 min, during which they received injections of either morphine (with dosage according to the aforementioned protocol) for the morphine group or an equivalent volume of saline for the control group, administered intraperitoneally. On day 4, during the retention experiment, the rats were released from the central section of the CPP apparatus and allowed to freely explore the two chambers for 15 min.

In the final testing phase, the same procedure was repeated for four consecutive days. CPP training was performed twice a day for 15 min each for 3 days and the CPP test was performed on the fourth day. The rats were again confined to the white box for 15 min, receiving the corresponding injections (morphine for the morphine group, with the final dose reaching 100 mg/kg, and an equivalent volume of saline for the control group). On day 14, during the test experiment, the rats were released from the central part of the CPP apparatus and allowed to explore the two chambers for 15 min.

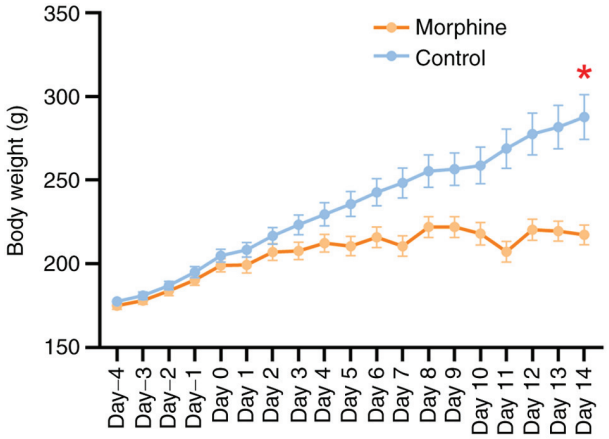

Figure 1. Body weight changes during morphine addiction modeling in rats. Body weight changes over the 14-day modeling period for rats in the morphine and control groups. On day 14, the average body weight of control rats was 233.6±8.0 g (maximum, 287.7 g), while that of rats in the morphine group was 206.2±3.4 g (maximum, 222.0 g). There was a statistically significant difference in the rate of body weight gain for rats in the morphine group compared with the control group (n=15, day 14, z=2.717, P=0.0057). *P<0.05.

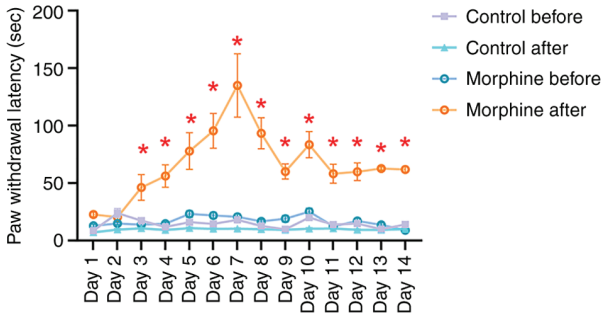

Figure 2. Changes in thermal nociceptive tolerance time during morphine addiction modeling in rats. Thermal nociceptive tolerance time over 14 days for control rats and rats in the morphine group. The gap in thermal nociceptive tolerance time before and after morphine injection was more pronounced in the morphine group compared with the control group before and after saline injection (n=15, U=74.4, P=0.039). *P<0.05.

Video behavior coding. During the 14-day morphine addiction model, rats were randomly selected from each of the two experiment groups (n=8 per group), and placed in a cage with one rat per cage. On day 15, video recordings were made and subsequently coded and analyzed using Observer XT software to quantify behavioral differences between the two groups.

The observed rats were divided into two batches of 8 (each with 4 addicted rats and 4 control rats), with one batch observed per day. Video recordings were made from 8:00 a.m. to 8:00 p.m. each day, with one rat per cage. A Xiaomi cloud platform video camera was temporarily placed in each rat cage, isolated from the rats by a wooden board, this setup allowed continuous recording of the rats' activity and behavior for 12 h, with the recorded video retained for subsequent analysis. Video coding and analysis were conducted using Observer XT software to examine differences in classical behaviors (such as eating, drinking, licking forelimbs, licking hind limbs, sleeping, walking and scratching) between the morphine group rats post-morphine addiction and the control group rats (45).

Statistical analysis. Data analysis was performed using GraphPad Prism (version 9; Dotmatics). The Wilcoxon rank-sum test and two-way ANOVA with Bonferroni correction were used to assess both experimental data and behavioral video coding recordings. P<0.05 was considered to indicate a statistically significant difference. Data are presented as mean ± SEM.

Results

Effect of continuous morphine administration on rat body weight. Following 14 days of escalating morphine doses, the weight difference between the morphine group and the control group showed a gradual and significant increase (day 14, z=2.717, P=0.0057, Fig. 1). After 14 days, compared with the control group, the weight growth rate of morphine group rats was lower. The final average body weight on day 14 was 206.2±3.4 g (maximum individual body weight, 222.0 g) for the morphine group. In contrast, the body weight of rats in the control group showed a steady increase, with a stable growth rate. The final average body weight on day 14 for the control group was 233.6±8.0 g (maximum individual body weight, 287.7 g). A difference between body weight in the two groups was evident from the first day, which increased over time. On day 14, the average body weight of control rats exceeded that of morphine-rats by 27.4 g with the greatest body weight difference between the two groups being 70.3 g.

Effect of morphine injection on thermal pain tolerance in rats. At 1.5 h post-intraperitoneal morphine injection, the morphine group exhibited a significantly extended duration of thermal pain tolerance compared with the same group pre-injection. There was no significant difference in thermal pain tolerance following saline injection in the control group. Ultimately, morphine-addicted rats demonstrated longer thermal tolerance than control rats (U=74.4, P=0.039, Fig. 2).

CPP experiments in morphine-addicted rats. CPP experiments were conducted on days 1-4 and days 11-14 for both the morphine and control groups. The analysis of CPP results involved recording the time spent by rats in the white box. The results before morphine addiction (day 4), demonstrated no statistically significant difference between the morphine and control groups (F=6.1., P=0.64, Fig. 3), indicating equivalent white box time for both groups.

In the experiment after morphine addiction (day 14), a significant difference was demonstrated between the morphine and control groups regarding time spent in the white box (F=5.7, P<0.001, Fig. 3), which demonstrated that morphine administration increased the time spent in the white box compared to saline administration, which validated the rat addiction model. Additionally, there was a significant difference in the white box time of morphine group rats before and after addiction (F=11.5, P<0.001, Fig. 3), further verifying the success of the morphine addiction model in rats.

Video coding analysis of the effects of morphine dependence on rat behavior. To record and statistically analyze the

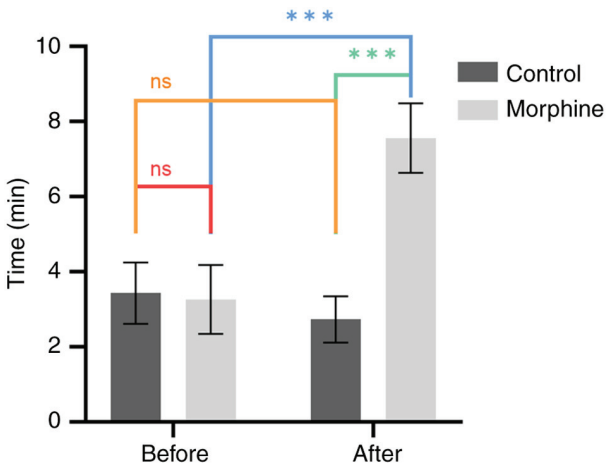

Figure 3. Comparison of white box time in CPP experiment before and after morphine addiction modeling. Comparative analysis of the time spent in the white box during the CPP experiment between rats in the morphine group and the control group. The comparisons include differences in white box time before morphine addiction (day 4) and after morphine addiction (day 14), and the variation in white box time for rats in the morphine group before and after addiction. Prior to morphine addiction (day 4), no significant difference was observed in the white box time between rats in the morphine group and the control group (n=10, χ^2=0.2, P=0.64). However, after morphine addiction (day 14), a significant difference in white box time was evident between the two groups (n=10, χ^2=134.8, P<0.001). Additionally, a significant difference in white box time was identified in the morphine group between day 4 and day 14 (n=10, χ^2=170.8, P<0.001). ***P<0.001. CPP, conditioned place preference; ns, not significant.

characteristic behaviors of rats after addiction, a 12-h video of rats in the morphine group and rats in the control group was recorded. The video was then coded and analyzed using Observer XT software, and the data was exported for analysis using the Mann-Whitney test to compare the statistical differences in the behaviors of the two groups. Compared with the control rats, the morphine group rats showed significant differences in drinking and licking the fore and hind limbs. They also exhibited characteristic behaviors such as an increased number ball sleeping, increased scratching, increased fur licking and changes in walking time at specific times of the day.

Regarding feeding, rats in the morphine group did not show significant differences compared with control rats (U=65.5, P=0.98, Fig. 4A). However, in terms of drinking, the morphine group drank significantly less water than the control group (U=17, P=0.0014, Fig. 4B). For the licking of the forelimbs, the morphine group licked significantly less compared with the control group (U=27, P=0.014, Fig. 4C). In contrast, for the licking of the hindlimbs, the morphine group licked significantly less than the control group (U=32, P=0.035, Fig. 4D). There was no significant difference between the two groups in terms of lifting the hindlimbs (U=62, P=0.082>0.05, Fig. 4E).

In the present study, 'marten sleeping' means sleeping curled up in the lateral arch of the rat with a clear view of the entire head. 'Ball sleeping' is when the rat is resting with its head curled up on its abdomen and its eyes closed. In the 12-h observation period, morphine-addicted rats displayed significantly more 'ball sleeping' behavior compared with control rats during the 2:30-3:00 p.m. period (z=-2.412, P=0.0159, Fig. 5A). There was no significant difference between the groups for 'marten sleeping' at any recorded time period. The frequency of scratching showed a statistically significant difference only during the 1:00-1:30 p.m. period (z=-1.964, P=0.0495; Fig. 5C). The frequency of licking showed a significant difference between the groups during the 3:30-4:00 p.m. period (z=-2.29, P=0.022, Fig. 5D). Additionally, the morphine group walked significantly less than the control group during the 12:00 a.m.-12:30 p.m. and 6:00-6:30 p.m. periods (z=-2.093, P=0.036; z=-2.039, P=0.0415; Fig. 5E). These results indicate that rats chronically injected with morphine exhibit characteristic behaviors such as increased ball sleeping, increased scratching, increased hair licking, and changes in walking behaviorat specific times of the day.

Discussion

Morphine addiction and dependence are recognized as neuropsychiatric diseases, involving various behavioral, neurobiological and molecular changes (55,71-75). In animal models, especially in rats, studying behavioral manifestations after morphine addiction is crucial for understanding the neural mechanisms of addiction and finding treatments. Video coding and analysis technology record animal behavior under specific conditions, using computer software to encode, identify and analyze the behavioral data. This technology enables comprehensive recording and accurate analysis of animal behavior, which is safe and non-invasive, and does not interfere with the animals' activities. Thus, it reveals the behavioral characteristics of rats after morphine addiction more accurately than traditional behavioral observation.

At present, the mechanism of morphine addiction has been studied and evaluated using various animal models, among which the rat model of chronic morphine administration is commonly used (48,49,51,76). In studies of rat or mouse behavior, most analyses use experiments to verify behavior, such as the rotarod, open field test, elevated plus maze test, cliff hanging, passive avoidance test, Morris water maze, light/dark box or light spot tests. The study of daily behaviors in rats mainly focuses on neuropathic pain and other diseases, using video coding analysis for assessment of daily walking gait (77-81). Research on addictive behavior primarily involves self-administration and CPP tests, with little use of daily behavior video coding analysis (82,83). However, after morphine administration, whether rats are addicted or not is usually judged by CPP test and self-administration experiment, and these methods have certain limitations and shortcomings, such as time-consuming, labor-intensive, requiring long-term behavioral training of animals and risk of infection and death due to invasive procedures. Therefore, in the present study, video coding was used to analyze the characteristic behavior of chronic morphine administration in rats, which was expected to provide a simple and quick method to analyze and evaluate morphine addiction through the behavioral changes before and after morphine addiction. Video behavior observation and analysis has many applications in studying animal behaviors such as pain, depression and anxiety. For example, Braw *et al* (84) demonstrated the depression and anxiety behavior of rats with different genetic models, finding differential expression of anxiety in pre-pubertal rats belonging to the 'depressed' strains, suggesting that these strains may be

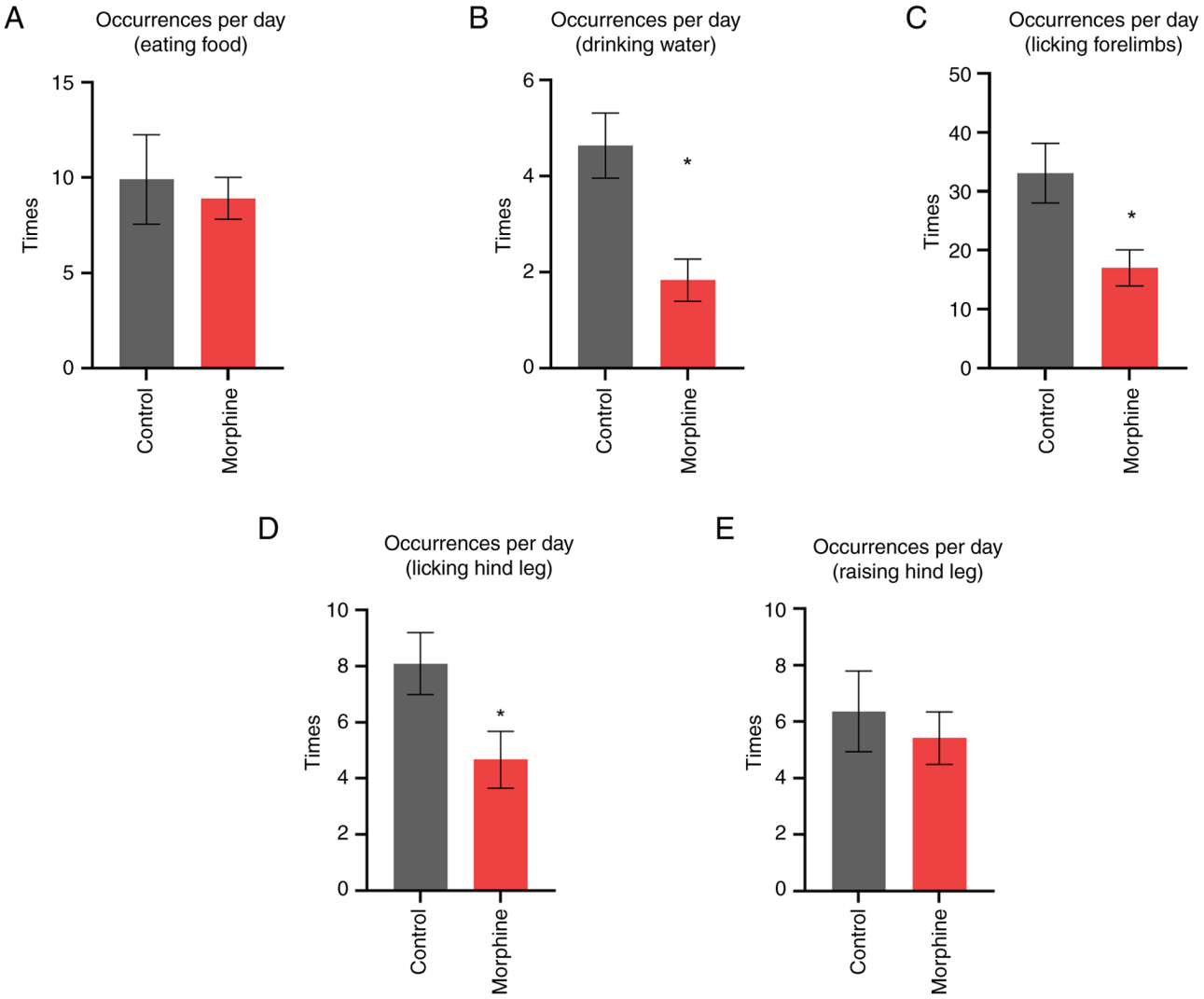

Figure 4. Behavioral analysis by video coding comparing rats in the morphine group and control group. (A) No significant difference was shown in feed intake between the morphine and control groups (n=8, U=65.5, P=0.98). (B) A significant difference was shown in water intake, with the morphine group drinking less than the control group (n=8, U=17, P=0.0014). (C) A significant difference was shown in foreleg licking, with the morphine group licking less than the control group (n=8, U=27, P=0.014). (D) A significant difference was shown in hind limb licking, with the morphine group licking more than the control group (n=8, U=32, P=0.035). (E) No significant difference was shown in hind leg lifting between the morphine and control groups (n=8, U=62, P=0.082>0.05). *P<0.05.

suitable for modelling different sub-groups of depression at young ages. Medvedev *et al* (85) demonstrated MK-801 and memantine acted against tactile allodynia induced by sciatic nerve ligatio. Yuan and Devine (86) demonstrated the self-injury behavior of rats induced by anxiety drugs, and finding the rats given anxiety-inducing drugs showed stronger self-injurious behavior. To the best of our knowledge, no previous studies have performed video behavioral coding analysis of morphine addiction behavior in rats. Therefore, using video coding analysis to study addiction behavior characteristics introduces innovative changes and has potential value for the researching of addiction mechanisms and treatments.

In the present study, weight observation, heat tolerance test, CPP test and behavioral video coding analysis were performed using a morphine addiction model in rats. The results indicated that morphine-addicted rats had increased heat pain tolerance and reduced weight gain. The present study used the method of video behavior coding analysis for the first time to find that rats with chronic morphine administration exhibited slower weight gain, smaller body size, and fragile fur (87). Behavioral changes were mainly characterized by decreased water intake, decreased toe licking and increased daytime sleep in a spherical posture. The present study analyzed various behavioral changes in rats before and after morphine addiction and provided a new method for verifying the morphine addiction rat model through behavioral video analysis (46-49,51,52).

Behavioral video analysis of morphine-addicted rats revealed significant differences in drinking and licking of the fore and hind limbs, as well as in sleeping postures and scratching during the middle of the day. According to Kon *et al* (88), morphine increases the expression of aquaporin-3 water channels in the colon by increasing the secretion of serotonin, which enhances water absorption from the lumen to the vasculature of the colon. Deroche *et al* (89) reported that opioids bind to MOP receptors in enteric

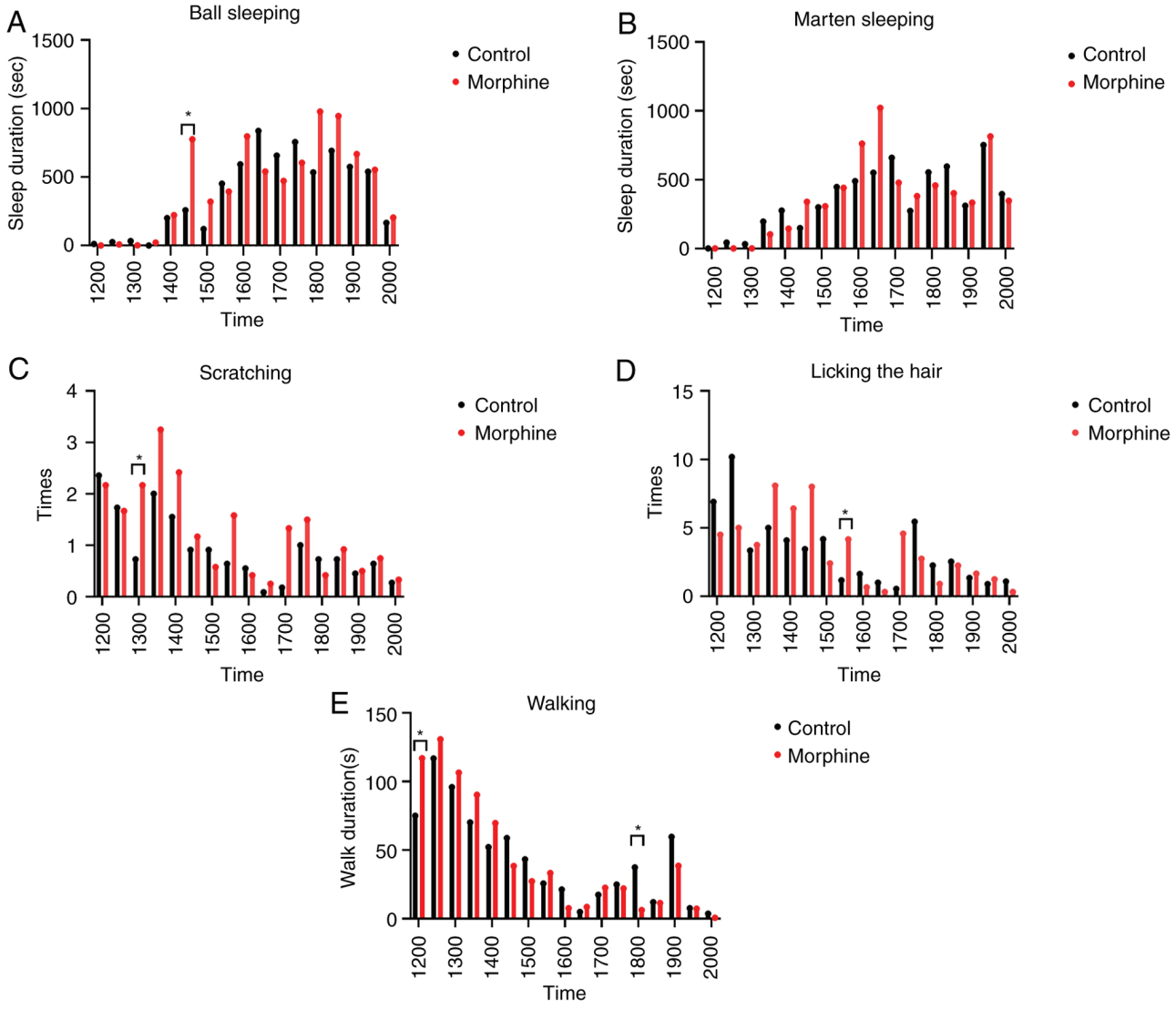

Figure 5. Behavioral comparison between morphine and control group rats over a 9-h period using video coding. (A) Comparison of 'ball sleeping' behavior, a significant difference was observed only during the 2:30-3:00 p.m. period (n=8, z=-2.412, P=0.0159). (B) Analysis of 'marten sleeping behavior (n=8, P=0.8>0.05). (C) Frequency of scratching actions, a significant difference was found only during the 1:00-1:30 p.m. period (n=8, z=-1.964, P=0.0495). (D) Frequency of fur licking, a significant difference was observed only during the 3:30-4:00 p.m. period (n=8, z=-2.29, P=0.022). (E) Comparison of walking frequency, significant differences were noted during the 12:00-12:30 p.m. (n=8, z=-2.093, P=0.036) and 6:00-6:30 p.m. periods (n=8, z=2.039, P=0.0415). *P<0.05.

neurons, delaying gastrointestinal transit time, and stimulating non-propulsive GI motility and the pylorus and ileocecal sphincters. Consequently, chronic morphine administration results in diminished thirst and reduced water excretion, leading to decreased water intake. Morphine group rats exhibited more frequent ball sleeping, especially during the 2:00-2:30 p.m. timeframe. The altered sleep posture suggests that long-term morphine exposure may lead to functional or structural changes in the limbic system and motor cortex, which are involved in mediating instinctive and emotional behaviors through Papez circuits (90). It is speculated that morphine affects the neurons and neural circuits in the limbic system or cortical nuclei, resulting in changes in sleep posture.

The present study analyzed the behavioral characteristics of morphine-addicted rats using the minimum sample size necessary to achieve statistical significance. Increasing the sample size could provide more precise behavior analysis and more convincing experimental conclusions. Additionally, the 14-day morphine addiction model is currently complex; future optimizations could make the model more efficient and reduce animal suffering.

In summary, high-resolution video equipment was used to record the behavior of morphine-addicted rats, and behavioral video coding and identification analysis were performed to systematically study the characteristics of morphine addiction behavior. By analyzing the behavioral characteristics of morphine-addicted rats, we can better understand the occurrence and development of addiction, reveal the impact of addiction on rat behavior and its potential neural mechanisms, and provide important theoretical and methodological support for further research on the neural circuit mechanisms of addiction and the development of related treatment strategies.

Acknowledgements

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 82073833) and the National Key R&D Plan (grant nos. 2022YFC3600500 and 2022YFC 3600502).

Availability of data and materials

The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

Authors' contributions

JY performed multiple experiments, acquired data and wrote the first draft of the manuscript. JY, TZ, DL, HL, XP, and FL performed data analysis. YZ, FX, and XW contributed to the study design and participated in revising the manuscript. YZ, FX, and XW confirmed the authenticity of all raw data and all authors agreed to be accountable for all aspects of the study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Experiments were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the ethics committee of Beijing Institute of Basic Medical Sciences (approval no. IACUC-DWZX-2022-715; Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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